Amendments to the Claims

Claim 1 (original): A method of analysis of amino acids, peptides or proteins, the method comprising:

(1) derivatizing a mixture of amino acids, peptides or proteins, to form at least one amino acid, peptide or protein derivatized to contain a fixed-charge ion, other than at the C-terminal or N-terminal end thereof;

(2) introducing the mixture of amino acids, peptides or proteins containing at least one amino acid, peptide or protein derivatized to contain a fixed-charge ion other than at the C-terminal or N-terminal end thereof, to a mass spectrometer;

(3) passing the mixture of amino acids, peptides or proteins containing at least one amino acid, peptide or protein derivatized to contain a fixed-charge ion, other than at the C-terminal or N-terminal end thereof, through a first mass resolving spectrometer to select precursor protein or peptide ions having a first mass-to-charge ratio;

(4) subjecting the precursor ions of the first mass-to-charge ratio to dissociation to form product ions having a second mass-to-charge ratio that is characteristic of a fragmentation occurring at a site adjacent to the fixed charge; and

(5) detecting the product ions having the second mass-to-charge ratio.

Claim 2 (original): The method of claim 1, wherein the product ions having the second mass-to-charge ratio are product ions formed by neutral loss of the fixed charge from the precursor ions.

Claim 3 (original): The method of claim 1, wherein the product ion having the second mass-to-charge ratio are product ions formed by charged loss of the fixed charge from the precursor ions.

Claim 4 (original): The method of claim 1, further comprising the step of:

(6) determining the identity of the derivatized peptide or protein.

Claim 5 (original): The method of claim 4, wherein the step of determining the identity of the derivatized peptide or protein is performed by first repeating steps (1), (2), (3) and (4) and then subjecting the product ions having the second mass-to-charge ratio to dissociation to form a series of product ions having a range of mass-to-charge ratios, for the purpose of determining the amino acid sequence of the peptide or protein.

Claim 6 (original): The method of claim 5, wherein the product ions having the second mass-to-charge ratio are formed by neutral loss from the precursor.

Claim 7 (original): The method of claim 5, wherein the wherein the product ion having the second mass-to-charge ratio is formed by charged loss from the precursor ion.

Claim 8 (original): The method of claim 4, wherein the step of determining the identity of the derivatized peptide or protein is performed by use of high resolution mass analyzers.

Claim 9 (original): The method of claim 8, wherein the use of high resolution mass analyzers provides mass accuracies of approximately 1-5 ppm on the product ion detected in step (5), or its complementary product ion.

Claim 10 (original): The method of claim 4, wherein the step of determining the identity of the derivatized peptide or protein comprises database searching to identify those peptides found to contain a fixed charge derivative.

Claim 11 (original): The method of claim 1, wherein the step of dissociation comprises a method selected from the group consisting of (i) collisions with an inert gas (collision-induced dissociation (CID) or collisionally-activated dissociation (CAD)); (ii) collisions with a surface (surface-induced dissociation (SID)); (iii) interaction with photons resulting in photodissociation, optionally using a laser; (iv) thermal/black body infrared radiative dissociation (BIRD); and (v) interaction with an electron beam, resulting in electron-induced dissociation for singly charged cations (EID), electron-capture dissociation (ECD) for multiply charged cations, or combinations thereof.

Claim 12 (currently amended): The method of claim 1 ~~any of claims 1-11~~, wherein the method is used for identification of amino acids, peptides or proteins.

Claim 13 (currently amended): The method of claim 1 ~~any of claims 1-11~~, wherein the method is used for quantitation of amino acids, peptides or proteins.

Claim 14 (currently amended): The method of claim 1 ~~any of claims 1-11~~, wherein the method is used for amino acid, peptide or protein differential quantitation based on the incorporation of suitable isotopic or structural labels to the fixed charge.

Claim 15 (currently amended): The method of claim 14, wherein the isotopic labels are ~~one or more~~ selected from the group consisting of ^{13}C, ^{15}N, and ^{2}H.

Claim 16 (currently amended): The method of claim 1 ~~any of claims 1-11~~, wherein the method is used for analysis of post translational modification status of amino acids, peptides or proteins.

Claim 17 (original): The method of claim 16, wherein the analysis of post translational modification status of amino acids, peptides or proteins comprises incorporation of the fixed-charge derivative via a β-elimination/Michael addition method for forming mass spectrometry stable derivatives of O-phosphorylated and O-glycosylated serine, or O-phosphorylated and O-glycosylated threonine.

Claim 18 (currently amended): The method of claim 1 ~~any of claims 1-11~~, wherein the method is used for analysis of cross-linking status of amino acids, peptides or proteins.

Claim 19 (currently amended): The method of claim 1 ~~of any of claims 1-11~~, wherein the method is used for analysis of interaction of proteins.

Claim 20 (currently amended): The method of claim 1 ~~any of claims 1-19~~, wherein the fixed-charge derivative is contained on the side-chain of a selected amino acid residue or a side-chain of a selected amino acid residue contained within a protein or peptide.

Claim 21 (original): The method of claim 20, wherein the selected amino acid residue is that of a rare amino acid.

Claim 22 (currently amended): The method of claim 20, wherein the selected amino acid residue contains a S atom ~~in the side chain thereof~~.

Claim 23 (original): The method of claim 22, wherein the amino acid residue is methionine, cysteine, homocysteine or selenocysteine.

Claim 24 (original): The method of claim 23, wherein the amino acid residue is methionine and wherein the method is performed according to any of Schemes 1, 3 and 4.

Claim 25 (original): The method of claim 23, wherein the amino acid residue is cysteine and wherein the method is performed according to any of Schemes 2, 5, 6, 7 and 8.

Claim 26 (original): The method of claim 20, wherein the selected amino acid residue is tryptophan or tyrosine.

Claim 27 (original): The method of claim 26, wherein the amino acid residue is tyrosine or tryptophan and wherein the method is performed according to any of Schemes 5 and 6.

Claim 28 (currently amended): The method of claim 20, wherein the ~~side chain~~ amino acid contains an S-alkyl group.

Claim 29 (original): The method of claim 28, wherein the amino acid residue is methionine, S-alkyl cysteine, S-alkyl homocysteine, S-alkyl tryptophan or S-alkyl tyrosine.

Claim 30 (currently amended): The method of claim 1 ~~any of claims 1-19~~, wherein the fixed-charge derivative is contained on a side-chain of a post-translationally modified amino acid residue.

Claim 31 (original): The method of claim 30, wherein the fixed-charge derivative is contained on an O-linked post-translationally modified amino acid residue.

Claim 32 (original): The method of claim 30, wherein the O-linked post-translationally modified amino acid residue is a dehydroalanine residue formed by β-elimination from an O-linked post-translationally modified serine amino acid residue (Scheme 9).

Claim 33 (original): The method of claim 30, wherein the O-linked post-translationally modified amino acid residue is a dehydroamino-2-butyric acid residue

formed by β-elimination from an O-linked post-translationally modified threonine amino acid residue.

Claim 34 (currently amended): The method of claim 1 ~~any of claims 1-19~~, wherein the fixed-charge derivative is contained on a cross-link contained between two amino acids, peptides or proteins (Scheme 10).

Claim 35 (currently amended): The method of claim 1 ~~any of claims 1-19~~, wherein the fixed-charge derivative is contained within a cross-linking reagent.

Claim 36 (currently amended): The method of claim 1 ~~any of claims 1-35~~, wherein the fixed-charge derivative is selectively pre-enriched by solid phase capture methods using fixed charge reagents covalently coupled to beads or insoluble polymers (Scheme 11).

Claim 37 (currently amended): The method of claim 1 ~~any of claims 1-36~~, wherein the fixed-charge ion is a sulfonium ion, a quaternary alkylammonium or a quaternary alkylphosphonium ion.

Claim 38 (currently amended): The method of claim 1 ~~any of claims 1-37~~, wherein the analysis of the amino acid, peptide or protein ion is performed by tandem mass spectrometry.

Claim 39 (original): The method of claim 38, wherein the tandem mass spectrometer is equipped with electrospray ionization (ESI) or matrix assisted laser desorption

ionization (MALDI) interfaces to transfer the protein or peptide ion into the gas-phase.

Claim 40 (original): The method of claim 38, wherein the tandem mass spectrometer is a tandem-in-space mass spectrometer, a tandem-in-time mass spectrometer, or a combination thereof.

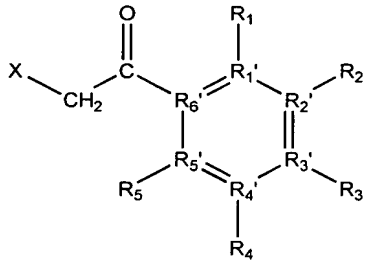
Claim 41 (original): The method of claim 40, wherein the tandem-in-space mass spectrometer is a sector mass spectrometer, a time of flight mass spectrometer, a triple quadrupole mass spectrometer, or a hybrid mass spectrometer combining time of flight and quadrupole instruments.

Claim 42 (original): The method of claim 41, wherein the sector mass spectrometer is a double focusing sector mass spectrometer or a hybrid mass spectrometer combining sector and quadrupole instruments.

Claim 43 (original): The method of claim 38, wherein the tandem-in-time mass spectrometer is a two-dimensional quadrupole ion trap mass spectrometer, a three-dimensional quadrupole ion trap mass spectrometer or a Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer.

Claim 44 (currently amended): The method of ~~any of the foregoing claims, wherein the method further includes~~ claim 1, further comprising one or more steps of protein extraction, protein separation, reduction and alkylation of cysteine disulfides and~~/or~~ digestion.

Claim 45 (currently amended): The method of claim 1 ~~any of the foregoing claims~~, wherein the amino acids, peptides or proteins are derivatized using a substituted acetophenone, or a salt thereof, or a solvate thereof, having the following formula:



Claim 46 (original): The method of claim 45, wherein X is any halogen, sulfonic ester, perchlorate ester or chlorosulfonate.

Claim 47 (original): The method of claim 45, wherein R_1-R_5 are H, and R_1' – R_6' are ^{12}C.

Claim 48 (original): The method of claim 45, wherein the substituted acetophenone is an isotopically encoded substituted acetophenone, or a salt thereof, or a solvate thereof.

Claim 49 (original): The method of claim 48, wherein X is any halogen, sulfonic ester, perchlorate ester or chlorosulfonate.

Claim 50 (original): The method of claim 49, wherein at least one of, and preferably at least three of R_1-R_5 are ^2H, and R_1'-R_6' are ^{12}C.

Claim 51 (original): The method of claim 49, wherein R_1-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C.

Claim 52 (currently amended): The method of claim 45 ~~any of claims 45-51~~, wherein at least one of R_1-R_5 is a functional group containing an atom other than hydrogen or carbon.

Claim 53 (currently amended): The method of claim 45 ~~any of claims 45-52~~, wherein the substituted acetophenone is water soluble.

Claim 54 (original): The method of claim 53, wherein

R_1 is SO_2H, and R_2-R_5 are H, and R_1'-R_6' are ^{12}C;

R_1 is H, R_2 is SO_2H, and R_3-R_5 are H, and R_1'-R_6' are ^{12}C;

R_{1-2} are H, R_3 is SO_2H, and R_4-R_5 are H, and R_1'-R_6' are ^{12}C;

R_1 is SO_3H, and R_2-R_5 are H, and R_1'-R_6' are ^{12}C;

R_1 is H, R_2 is SO_3H, and R_3-R_5 are H, and R_1'-R_6' are ^{12}C; or

R_{1-2} are H, R_3 is SO_3H, and R_4-R_5 are H, and R_1'-R_6' are ^{12}C.

Claim 55 (original): The method of claim 53, wherein

R_1 is SO_2H, and at least one of, and preferably at least three of R_2-R_5 are ^2H, and R_1'- R_6' are ^{12}C;

R_1 is SO_2H, and R_2-R_5 are H, and at least one of, and preferably at least three of R_1'- R_6' are ^{13}C;

R_2 is SO_2H, and at least one of, and preferably at least three of R_1 and R_3-R_5 are ^2H, and R_1'-R_6' are ^{12}C;

R_2 is SO_2H, and R_1 and R_3-R_5 are H, and at least one of, and preferably at least three of R_1'- R_6' are ^{13}C;

R_3 is SO_2H, and at least one of, and preferably at least three of R_1-R_2 and R_4-R_5 are ^{2}H, and R_1'- R_6' are ^{12}C;

R_3 is SO_2H, and R_1-R_2 and R_4-R_5 are H, and at least one of, and preferably at least three of R_1'- R_6' are ^{13}C;

R_1 is SO_3H, and at least one of, and preferably at least three of R_2-R_5 are ^{2}H, and R_1'- R_6' are ^{12}C;

R_1 is SO_3H, and R_2-R_5 are H, and at least one of, and preferably at least three of R_1'- R_6' are ^{13}C;

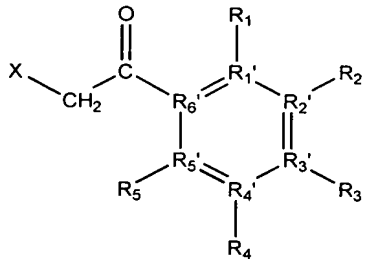
R_2 is SO_3H, and at least one of, and preferably at least three of R_1 and R_3- R_5 are ^{2}H, and R_1'- R_6' are ^{12}C;

R_2 is SO_3H, and R_1 and R_3-R_5 are H, and at least one of, and preferably at least three of R_1'- R_6' are ^{13}C;

R_3 is SO_3H, and at least one of, and preferably at least three of R_1-R_2 and R_4-R_5 are ^{2}H, and R_1'- R_6' are ^{12}C; and

R_3 is SO_3H, and R_1-R_2 and R_4-R_5 are H, and at least one of, and preferably at least three of R_1'-are ^{13}C.

Claim 56 (currently amended): A substituted acetophenone, or a salt thereof, or a solvate thereof, having the following formula:



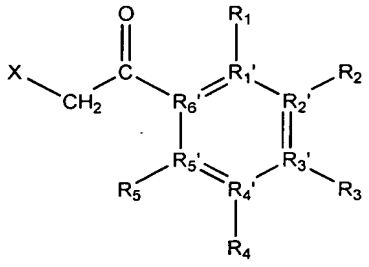
wherein X is a sulfonic ester, perchlorate ester or chlorosulfonate, R_1-R_5 are H and R_1'-R_6' are selected from the group consisting of ^{12}C and ^{13}C, or R_1-R_5 are ^{2}H and R_1'-R_6' are ^{13}C.

Claim 57 (cancelled)

Claim 58 (original): The substituted acetophenone of claim 56, wherein the substituted acetophenone is an isotopically encoded substituted acetophenone, or a salt thereof, or a solvate thereof.

Claims 59-60 (cancelled)

Claim 61 (currently amended): A water soluble substituted acetophenone, or a salt thereof, or a solvate thereof, having the following formula:



wherein X is Br or I, R_1-R_5 are H and R_1'-R_6' are selected from the group consisting of ^{12}C and ^{13}C, or R_1-R_5 are ^{2}H and R_1'-R_6' are ^{13}C.

Claim 62 (cancelled)

Claim 63 (currently amended): The water soluble substituted acetophenone of claim 61-~~62~~, wherein X is Br or I.

Claim 64 (cancelled)

Claim 65 (original): An isotopically encoded form of the water soluble substituted acetophenone of claim 61.

Claim 66 (currently amended): The isotopically encoded water soluble substituted acetophenone of claim 61-~~64~~, wherein X is any halogen, sulfonic ester, perchlorate ester or chlorosulfonate.

Claim 67 (original): The isotopically encoded water soluble substituted acetophenone of claim 66, wherein

R_1 is SO_2H, at least one of, and preferably at least three of R_2 - R_5 are ^{2}H, and R_1'-R_6' are ^{12}C;

R_1 is SO_2H, R_2-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C;

R_2 is SO_2H, at least one of, and preferably at least three of R_1 and R_3-R_5 are ^{2}H, and R_1'-R_6' are ^{12}C;

R_2 is SO_2H, R_1 and R_3-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C;

R_3 is SO_2H, at least one of, and preferably at least three of R_1-R_2 and R_4- R_5 are ^{2}H, and R_1'-R_6' are ^{12}C;

R_3 is SO_2H, R_1-R_2 and R_4 - R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C;

R_1 is SO_3H, at least one of, and preferably at least three of R_2 - R_5 are ^{2}H, and R_1'-R_6' are ^{12}C;

R_1 is SO_3H, R_2-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C;

R_2 is SO_3H, at least one of, and preferably at least three of R_1 and R_3-R_5 are ^{2}H, and R_1'-R_6' are ^{12}C;

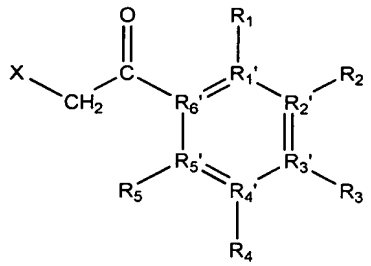
R_2 is SO_3H, R_1 and R_3-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C;

R_3 is SO_3H, at least one of, and preferably at least three of R_1-R_2 and R_4- R_5 are ^{2}H, and R_1'-R_6' are ^{12}C;

R_3 is SO_3H, R_1-R_2 and R_4-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C.

Claim 68 (currently amended): A reagent kit for analysis of amino acids, peptides or proteins by mass spectrometry comprising a container containing the substituted acetophenone of claim 56 ~~any of claims 56-67~~.

Claim 69 (original): A reagent kit for analysis of amino acids, peptides or proteins by mass spectrometry comprising a container containing a substituted acetophenone, or a salt thereof, or a solvate thereof, having the following formula:



wherein X is a halide.

Claim 70 (original): The reagent kit of claim 69, wherein X is Br or I.

Claim 71 (currently amended): The reagent kit of claim 69 ~~or 70~~, wherein R_1-R_5 are H, and R_1'–R_6' are ^{12}C.

Claim 72 (original): The reagent kit of claim 69, wherein the substituted acetophenone is an isotopically encoded substituted acetophenone, or a salt thereof, or a solvate thereof.

Claim 73 (original): The reagent kit of claim 72, wherein at least one of, and preferably at least three of R_1-R_5 are ^2H, and R_1'–R_6' are ^{12}C.

Claim 74 (original): The reagent kit of claim 72, wherein R_1-R_5 are H, and at least one of, and preferably at least three of R_1'-R_6' are ^{13}C.

Claim 75 (currently amended): The reagent kit of claim 69 ~~68-74~~, further comprising one or more containers containing: cysteine disulfide reducing agents, cysteine alkylating reagents, proteases or chemical cleavage agents, and solvents.

Claim 76 (original): The reagent kit of claim 75, wherein the cysteine disulfide reducing agents are: dithiothreitol (DTT), β-mercaptoethanol, tris-carboxyethyl phosphine (TCEP), and/or tributylphosphine (TBP).

Claim 77 (original): The reagent kit of claim 75, wherein the cysteine alkylating reagents are alkylhalides (e.g. iodoacetic acid, iodoacetamide), vinylpyridine or acrylamide.

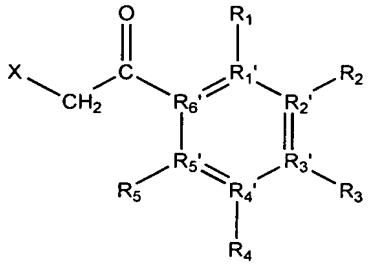
Claim 78 (original): The reagent kit of claim 75, wherein the proteases or chemical cleavage agents are trypsin, Endoproteinase Lys-C, Endoproteinase Asp-N, Endoproteinase Glu-C, pepsin, papain, thermolysin, cyanogen bromide, hydroxylamine hydrochloride, 2-[2'-nitrophenylsulfenyl]-3-methyl-3'-bromoindole (BNPS-skatole), iodosobenzoic acid, pentafluoropropionic acid and/or dilute hydrochloric acid.

Claim 79 (original): The reagent kit of claim 75, wherein the solvents are urea, guanidine hydrochloride, acetonitrile, methanol and/or water.

Claim 80 (original): An amino acid or peptide comprising an amino acid derivatized to include a side chain fixed-charge sulfonium ion, quaternary alkylammonium ion or quaternary alkylphosphonium ion.

Claim 81 (cancelled)

Claim 82 (original): The amino acid or peptide of claim 80, wherein the amino acid is derivatized using a substituted acetophenone, or a salt thereof, or a solvate thereof, having the following formula:



wherein X is a halide.

Claim 83 (original): The amino acid or peptide of claim 82, wherein X is Br or I.

Claim 84 (currently amended): The amino acid or peptide of claim 82 ~~or 83~~, wherein R_1-R_5 are H, and R_1'–R_6' are ^{12}C or at least one of R_1'-R_6' is ^{13}C, or R_1-R_5 are ^{2}H and R_1'–R_6' are ^{12}C.

Claim 85 (original): The amino acid or peptide of claim 82, wherein the substituted acetophenone is an isotopically encoded substituted acetophenone, or a salt thereof, or a solvate thereof.

Claims 86-87 (cancelled)

Claim 88 (currently amended): The amino acid or peptide of claim 80 ~~any of claims 80-87~~, wherein the amino acid derivative is isotopically encoded.

Claim 89 (currently amended): A method for providing an internal standard in a mass spectrometer method comprising adding to a sample a predetermined quantity of the fixed charge derivatized amino acid or peptide of claim 80 ~~claimed in claim 80-88~~.

Claim 90 (new): A reagent kit for analysis of amino acids, peptides or proteins by mass spectrometry comprising a container containing the substituted acetophenone of claim 61.